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Reduction in Focal Cerebral Ischemia by Agents Acting at Imidazole Receptors

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Summary: Treatment with the α_2-adrenergic antagonist idazoxan (IDA) can provide protection from global cerebral ischemia. However, IDA also recognizes another class of receptors, termed imidazole (IM) receptors, which differ from α_2-adrenergic receptors and are responsible for the hypotensive actions of some centrally acting agents such as the oxazole rilmenidine (RIL). We therefore sought to determine whether RIL, an agent highly selective for IM receptors, offered protection from focal cerebral ischemia elicited in rat by ligation of the middle cerebral artery (MCA). We compared the effects of RIL with the effects of IDA and the selective non-IM α_2-antagonist SKF 86466 (SKF). In addition, we examined whether the neuroprotective effects of RIL and IDA could be attributed to changes in local CBF (LCBF). The MCA was occluded and animals either received immediate administration of drug while arterial pressure was maintained for 1 h or had local CBF increased to 200% of control for 1 h by hypercapnia or hypertension. RIL elicited a significant dose-dependent preservation of tissue to 33% of control at optimal dose (0.75 mg/kg). IDA (3 mg/kg) significantly reduced the size of ischemic infarction by 22%. In contrast, SKF (15 mg/kg) as well as doubling of LCBF did not preserve ischemic tissue. We conclude that both RIL and IDA can reduce focal ischemic infarction but that the mechanism does not appear secondary to antagonism of α_2-adrenergic receptors or elevation of LCBF. Occupation of IM receptors, either in the ischemic zone or at remote brain sites, may be responsible for neuroprotection of RIL and IDA. Key Words: Adrenergic receptor—Focal cerebral ischemia—Idazoxan—Imidazole receptor—Rilmenidine.

It has been proposed that stimulation of adrenergic receptors in brain may offer protection from neuronal damage associated with cerebral ischemia (Koide et al., 1986; Wieloch et al., 1986). In partial support of the hypothesis are the findings that treatment with the drug idazoxan {2-[2-(1,4-benzodioxanyl)]-2-imidazoline HCl; IDA}, an agent usually considered to be an α_2-adrenergic antagonist, reduces the magnitude of the delayed neuronal degeneration elicited by global cerebral ischemia (Yasuda et al., 1978; Gustafson et al., 1989, 1990).

However, over the last several years, evidence has accrued to indicate that IDA also recognizes another class of receptors that bind substituted imidazolines such as clonidine and its congeners (Michel et al., 1989). These receptors, termed imidazole (IM), IM-preferring or imidazoline-guanidinium receptors (Ernsberger et al., 1987; Coupry et al., 1989; Lehmann, 1989), differ from the α_2-adrenergic receptors with respect to anatomical distribution in brain and periphery (Ernsberger et al., 1987; Bricca et al., 1989*b*; Michel et al., 1989; Wickberg and Uhlen, 1990), in signal transduction mechanisms (Ernsberger et al., 1988*a*; Regunathan et al., 1990), and in their very distinct binding profiles, which exclude native adrenergic ligands such as norepinephrine (Ernsberger et al., 1987, 1988*a*). Whether IDA acts as an agonist or antagonist at IM receptors is not known. Conceivably the neuroprotective actions of IDA may relate more to its interactions with IM than with α_2-adrenergic receptors in brain.

In the present study we have therefore examined the neuroprotective effects of an agent with high

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Abbreviations used: IDA, idazoxan; IM, imidazole; LCBF, local CBF; LDF, laser-Doppler flowmetry; MCA, middle cerebral artery; RIL, rilmenidine; SHR, spontaneously hypertensive rat; SKF, SKF 86466.

selectivity for IM receptors, the oxazole rilmenidine [2-(dicyclopropylmethyl)amino-2-oxazoline phosphate; RIL] (Gomez et al., 1991). We sought to determine whether the agent offered protection against focal cerebral ischemia elicited in rat by ligation of the middle cerebral artery (MCA), if so whether the effect was mimicked by IDA, which has yet to be tested against focal ischemia, and finally whether any neuroprotective effects of the agent can be attributed to changes in local CBF (LCBF). We demonstrate that RIL elicits a dose-dependent reduction in the size of the focal ischemic infarction produced by occlusion of the MCA in rat, that the effect is mimicked by IDA, but that administration of the highly selective α_2-adrenergic antagonist SKF 86466 (SKF) or increasing LCBF by 200% will not reduce the size of infarction. The results suggest that the neuroprotective effects of RIL and IDA may relate to occupation of IM receptors.

METHODS

Methods for production of focal cerebral infarction by occlusion of the MCA, for computation of infarct volume, and for recording of LCBF by laser-Doppler flowmetry (LDF) have been described in detail in prior publications (Iadecola and Reis, 1990; Reis et al., 1991) and will only be summarized here.

General procedures

Studies were performed on 55 adult male Wistar-Kyoto rats of the spontaneously hypertensive strain (SHR) weighing 280–370 g. SHRs were selected because of the uniformity of lesions elicited between animals (Brint et al., 1988; Reis et al., 1991). Animals were maintained in a 20°C enviroment and fed laboratory chow ad libitum. They were anesthetized with isoflurane (1–3% in 100% O_2) blown over the nose. Thin-wall polyethylene (1.3-mm OD) catheters were placed in the right femoral artery and vein. The arterial catheter was connected to a Statham P23 db transducer for continuous monitoring of arterial pressure and heart rate on a chart recorder (Beckman model R611). The venous catheter was employed for drug administration.

Following instrumentation, rats were mounted in a Kopf stereotaxic apparatus and maintained within 37–38°C by a thermostatically controlled infrared lamp connected to a rectal probe. Blood (0.2 ml) was withdrawn from the arterial catheter for determination of pH, Po_2, and Pco_2 by a blood gas analyzer (Ciba-Corning model 178) and for calculation of hematocrit. Throughout the duration of the experiment, which required 3 h, the animals remained in an anesthetized state with isoflurane.

The MCA was exposed for occlusion distal to the lenticulostriatal branches by a method modified from that of Tamura et al. (1981). Animals were rotated in the stereotaxic frame to a lateral position with the right side facing upward. The skin was incised midway between the orbit and external auditory meatus and the temporal muscle retracted. Under magnification, the MCA was exposed through a hole in the skull at a point rostral to the fusion of the zygomatic arch and squamous bone ma a dental drill continuously cooled with a sali The dura overlying the MCA was incised. A sn hook was inserted under the MCA just superio inferior cortical vein and the artery was subs cauterized.

Immediately following MCA occlusion, drug ministered intravenously with arterial pressur tained in the control range, if necessary, with phe rine. After 1 h, the cranial wound was covered foam and closed with sutures. Wounds were trea 1% lidocaine and Bacitracin ointment. The femor and venous catheters were capped, the animal moved from the stereotaxic frame, anesthesia v continued, and the animal was returned to its ca

Twenty-four hours later, animals were reanest with isoflurane and killed by decapitation. The were removed and placed in liquid Freon at Brains were serially sectioned at 20 μm on a c microtome, at −20°C, in the coronal plane. Sam tions were obtained every 200 μm and stained Nissl method.

Each section was identified by reference to sponding levels in an atlas of the rat brain (Paxir Watson, 1986). Microscopically, ischemic nerve c was characterized by loss of Nissl bodies, cell b traction, and hyperchromasia of the cell nucleus. third brain section (600 μm) was projected on a dat from an overhead projector. The area of infarcti computed by tracing the lesion corresponding to t of Nissl substance on a Jandel Scientific Sigma Sca volume of the infarct was computed as the product cross-sectional area (mm^2) for all sections and t tance between sections, and then plotted as a func the distance from the interaural line.

In some experiments LCBF was increased diffu assess the effects of elevated LCBF on the size infarction. LCBF was increased in two ways: by ing MABP above the autoregulated range for (Barry et al., 1982) with infusion of phenylephrine hypercapnia. Changes in LCBF were monitored c lateral to the site of MCA occlusion by LDF, whic mits continuous recording of LCBF in vivo (Dirr al., 1989). Hypercapnia was induced by administeri CO_2 with 100% O_2 and phenylephrine was used to i hypertension. Elevated CBF was maintained for 1 h lowing this period, wounds were closed and asep treated with lidocaine 1% and Bacitracin; CO_2 or ylephrine administration was discontinued and th mals were returned to their cages.

In studies in which LCBF was measured, LDI performed with a PeriFlux PF3 (Perimed) flowmete flow values expressed as perfusion units and chan LCBF expressed as percentage change in flow. A h mm in diameter was drilled through the parietal bor mm lateral and 2.5 mm caudal to the bregma just pr ligation of the MCA. The dura was left intact. Follc the application of mineral oil to the hole, the probe (c probe, model PF 316) was positioned ~0.5 mm abov dural surface. Care was taken to avoid bleeding fro bone or dura as well as exposure of the probe to c light since these can reduce the flow signal. At tim was necessary to reposition the probe 0.3 mm rostra caudally to avoid placement over large pial vessels (μm), which invalidate the LDF flow signal (Haberl e 1989). The analog output from the LDF was displaye

a polygraph (Beckman model R611). A time constant of 3 s was employed to avoid pulsatile variations in flow signal.

Studies were conducted in groups with n = 5 rats. The groups consisted of (a) rats in which only the MCA was occluded (MCA control); (b) rats receiving intravenous drugs RIL (0.05, 0.25, 0.50, 0.75, 1.0, and 2.0 mg/kg), IDA (3 mg/kg), or SKF (15 mg/kg); (c) rats in which LCBF was increased by hypercapnia or hypertension. MABP, arterial blood gases, and hematocrit did not differ between groups (except for MABP and Pco_2 in hypertensive or CO_2-treated rats) (see Table 2). Each animal group underwent treatment as a discrete entity. Subsequent infarct volume computation was randomly ordered among the animal groups. Physiological variables, LCBF, infarct cross-sectional area, and infarct volume are expressed as means ± SD. Comparisons of infarct volume as well as physiological parameters among groups were assessed using the analysis of variance and the Newman-Keuls test. Differences were considered significant for $p < 0.05$.

RESULTS

MCA occlusion in control animals

Occlusion of the MCA in the control group (n = 5) resulted in a well-defined infarction within the vascular territory perfused by the MCA in rat (Fig. 1). When expressed as an area of infarction along the rostrocaudal plane, the lesion began at the temporal cortex (3.75 mm anterior to the interaural line) and extended to the frontal and angular insular cortex (14.25 mm anterior to the interaural line) for a total of 10.5 mm (Fig. 1). Laterally the lesion involved large portions of the parietal, granular insular, angular insular, pyriform, perirhinal, and temporal cortices (Fig. 2). Medially, the infarct involved the lateral caudate and putamen, portions of the hippocampal formation, and the dorsal portion of the amygdala (Fig. 2). The lesions were remarkably similar between animals as demonstrated by the uniform distribution and small variability among the cross-sectional areas at comparable sites (Fig. 1). The maximal cross-sectional area of the infarct was 31.65 ± 2.61 mm^2 at 8.0 mm anterior to the interaural line. The average volume was 196.31 ± 14.86 mm^3 (Table 1).

Effects of RIL on focal ischemia

The MCA was occluded in five animals immediately followed by administration of a bolus of RIL (1 mg/kg i.v.). This dose of RIL is at the upper end of the dose-response curve for the hypotensive actions of the drug and, as reported elsewhere (Gomez et al., 1991), lowers MABP by ~45 mm Hg with a maximum response occurring in 15 min in rats of the Sprague-Dawley strain. To counteract the hypotension, MABP was maintained in the control range by a variable infusion of phenylephrine (0.5–6.5 μg/min) beginning at the time of RIL administration and maintained for the 1 h following vascular occlusion. MABP did not vary from control following completion of phenylephrine treatment (Table 2).

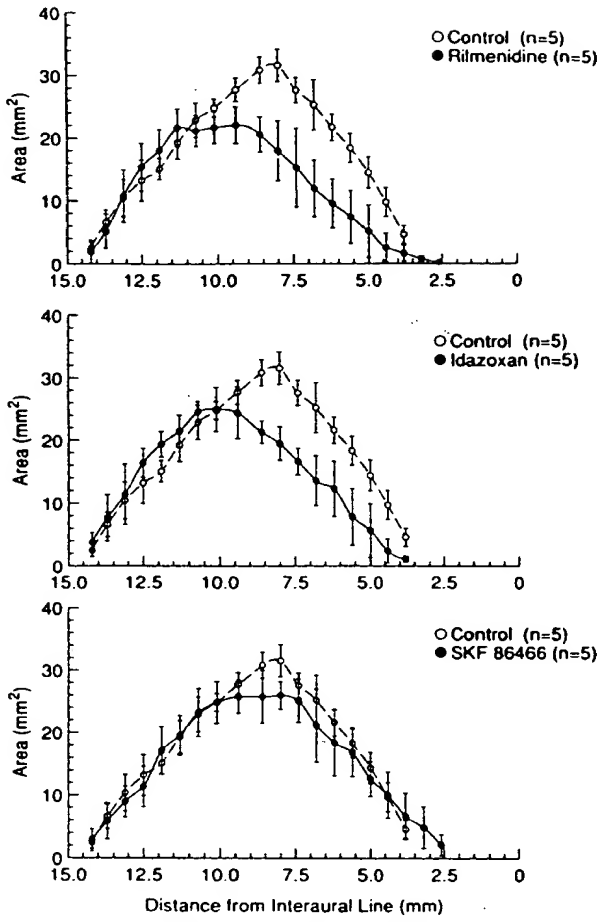

FIG. 1. Area of focal infarction (mm^2) at progressive distances (mm) from the interaural line 24 h after middle cerebral artery occlusion in control animals (○; n = 5) and animals treated with rilmenidine (●; RIL; 1.0 mg/kg; n = 5), idazoxan (●; IDA; 3.0 mg/kg; n = 5), and SKF 86466 (●; SKF; 15.0 mg/kg; n = 5). Maximal cross-sectional area of infarction was 8.0 mm anterior to the interaural line in the control and SKF groups. In contrast, preservation of ischemic tissue following treatment with either RIL or IDA was confined to the caudal portions of the lesion (maximum cross-sectional area of infarction 10.0 mm anterior to the interaural line).

RIL at this dose reduced the size of the infarction elicited by MCA occlusion (Fig. 1; Table 1). While the rostrocaudal extent of the lesion was comparable between treated and untreated rats (Fig. 1), the cross-sectional area of infarction was reduced in the caudal two-thirds of the infarction. The maximum cross-sectional area was 22.12 ± 2.94 mm^2, or ~60% of control, and located 10.0 mm anterior to the interaural line (Fig. 1). The volume of the infarct was reduced by ~29% as compared with controls (Table 1).

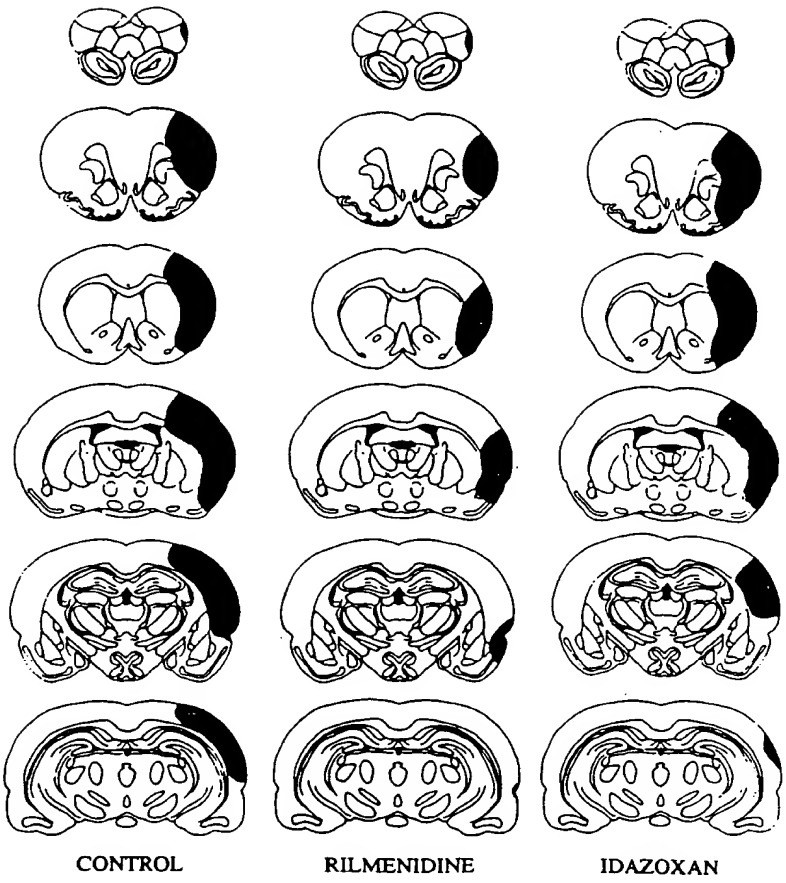

FIG. 2. Representative serial coronal sections of distribution of ischemic infarct produced by middle cerebral artery (MCA) occlusion in control (n = 5), rilmenidine-treated (1.0 mg/kg; n = 5), and idazoxan-treated (3.0 mg/kg; n = 5) animals. The dark region represents infarct area 24 h following MCA occlusion for each group. When compared with control, areas of preservation by either rilmenidine or idazoxan treatment included the hippocampal formation, perirhinal cortex, ventral and dorsal portions of the parietal cortex, temporal cortex, frontal cortex, as well as medial portions of the caudate and putamen.

The distribution of the lesion involved the parietal cortex, granular insular cortex, angular insular cortex, caudate-putamen, and pyriform cortex (Fig. 2). Areas of preservation, when compared with control, included the hippocampal formation, amygdala, perirhinal cortex, ventral and dorsal portions of the parietal cortex, granular insular cortex, angular insular cortex, temporal cortex, and frontal cortex. In addition, medial portions of the caudate and putamen were spared.

Dose response of RIL

To determine whether the neuroprotective actions of RIL were dose dependent, RIL was admin-

TABLE 1. *Maximum cross-sectional area, infarct volume, and percentage difference fron control in rats treated with rilmenidine, idazoxan, SKF 86466, and elevated CBF by either hypercapnia or hypertension*

Group	Maximum cross-sectional area (mm^2)	Infarct volume (mm^3)	% change from control	p
Control	31.65 ± 2.61	196.31 ± 14.86	0	—
Rilmenidine				
0.05 mg/kg i.v.	29.28 ± 3.34	187.90 ± 13.39	4	NS
0.25 mg/kg i.v.	30.48 ± 4.46	201.61 ± 32.97	−3	NS
0.50 mg/kg i.v.	29.34 ± 3.28	176.55 ± 10.73	10	NS
0.75 mg/kg i.v.	22.18 ± 2.12	132.09 ± 17.51	33	<0.05
1.00 mg/kg i.v.	22.12 ± 2.94	138.86 ± 17.47	29	<0.05
2.00 mg/kg i.v.	34.03 ± 3.45	242.36 ± 21.78	−19	<0.05
Idazoxan				
3 mg/kg i.v.	25.05 ± 3.56	152.96 ± 10.07	22	<0.05
SKF 86466				
15 mg/kg i.v.	26.06 ± 2.23	185.95 ± 24.22	5	NS
CO_2 treated	36.98 ± 6.72	201.35 ± 45.54	−3	NS
Phenylephrine treated	32.71 ± 6.95	203.96 ± 35.10	−4	NS

TABLE 2. *MABP, Pco_2, Po_2, pH, and hematocrit among control and treated animals*

Group	MABP (mm Hg)	Pco_2 (mm Hg)	Po_2 (mm Hg)	pH	Hematocrit (%)
Control	153 ± 26	40.5 ± 7.3	459 ± 33	7.36 ± 0.03	38 ± 3
Rilmenidine					
0.05 mg/kg i.v.	155 ± 18	40.0 ± 4.9	481 ± 23	7.35 ± 0.12	38 ± 4
0.25 mg/kg i.v.	149 ± 11	41.3 ± 6.4	444 ± 31	7.36 ± 0.10	39 ± 2
0.50 mg/kg i.v.	150 ± 24	37.1 ± 5.7	460 ± 20	7.39 ± 0.07	38 ± 3
0.75 mg/kg i.v.	147 ± 10	36.1 ± 6.2	466 ± 42	7.40 ± 0.04	38 ± 2
1.00 mg/kg i.v.	150 ± 15	39.2 ± 6.2	475 ± 37	7.39 ± 0.08	39 ± 2
2.00 mg/kg i.v.	143 ± 34	36.1 ± 5.9	479 ± 33	7.41 ± 0.04	39 ± 3
SKF 86466					
15 mg/kg i.v.	144 ± 11	39.3 ± 7.6	431 ± 25	7.39 ± 0.01	40 ± 2
Idazoxan					
3 mg/kg i.v.	146 ± 21	40.3 ± 6.0	450 ± 15	7.35 ± 0.06	38 ± 3
CO_2 treated	159 ± 8	66.2 ± 18.5[a]	335 ± 49[a]	7.29 ± 0.09	40 ± 4
Phenylephrine treated	189 ± 20[a]	39.8 ± 5.0	472 ± 29	7.36 ± 0.02	41 ± 2

Parameters were not significantly different among animal groups except in the hypercapnic group with Pco_2 = 66.2 ± 8.2 mm Hg and the hypertensive group with MABP = 189 ± 9 mm Hg. n = 5 for all groups.
[a] Difference from control ($p < 0.05$).

istered in different doses (0.05, 0.25, 0.5, 0.75, and 2 mg/kg) to groups of five rats immediately following MCA occlusion. RIL at 0.05 and 0.25 mg/kg did not alter MABP. At 0.5, 0.75, and 2 mg/kg the hypotensive effects were progressively greater, requiring larger doses of phenylephrine (0.5–10.0 μg/min) to maintain MABP in the control range during the 1 h following administration of RIL. Upon completion of phenylephrine, MABP returned to control levels except in the 2-mg/kg group. In this group, MABP was reduced to ~75 mm Hg, a 50% drop in MABP from control at the time the animals were returned to their cages.

The magnitude of neuroprotection elicited by RIL was dose related (Fig. 3, left). RIL at 0.05 and 0.25 mg/kg had no effect upon the distribution and magnitude of the infarction produced by MCA occlusion (Table 1). RIL at 0.5 mg/kg insignificantly reduced infarction volume by ~10% (Table 1). However, RIL at 0.75 mg/kg significantly reduced infarct volume ($p < 0.05$) by ~33% (Table 1). The volume of preservation was not increased with RIL 1.0 mg/kg (Fig. 3; Table 1).

Interestingly, the size of the infarct was significantly increased by 19% with RIL at 2.0 mg/kg (Fig. 3; Table 1). As in the control group, the lesion involved the frontal cortex, angular insular cortex, parietal cortex, piriform cortex, granular insular cortex, perirhinal cortex, portions of the hippocampal formation, and the caudate-putamen. However, the infarct also extended rostrally to the orbital cortex, medially to the corpus callosum and significant portions of the caudate-putamen, and caudally to the occipital cortex. The increase in infarct size may be secondary to the associated hypotension. Irrespective of cause, this finding indicates that the infarction produced by MCA occlusion in the rat is not at a "ceiling" and is capable of being enlarged.

Effects of IDA on focal ischemia

IDA (3 mg/kg) was administered by bolus intravenous injection to five rats immediately following MCA occlusion with blood pressure maintained by phenylephrine (0.5–6.5 μg/min) (Table 2). This amount of IDA is comparable with the dose used with prolonged infusion of IDA that has been shown to reduce the neuronal necrosis elicited by global cerebral infarction in rat (Gustafson et al., 1989). After 1 h and at the termination of the infusion of phenylephrine, MABP did not differ from control.

IDA, like RIL, significantly reduced the size of the infarction (Fig. 1). In the IDA group, the reduction of the lesion produced by MCA occlusion was comparable in distribution to that produced by the optimal dose of RIL, with the maximal area of salvage occurring in the caudal two-thirds of the lesion (Figs. 1 and 2). Although the magnitude of the reduction was 22% of control, this was slightly but not significantly less than the 33% salvage seen with RIL (Table 1).

Effects of SKF

The selective α_2-adrenergic antagonist SKF (Hieble et al., 1986) was administered intravenously as a bolus (15 mg/kg) immediately following occlusion of the MCA. Phenylephrine (0.5–6.5 μg/min) was administered following the bolus for 1 h to maintain MABP in the control range. MABP did not differ from control following discontinuation of phenylephrine (Table 2). SKF had no effect upon the size or distribution of the infarction elicited by MCA occlusion (Fig. 1; Table 1).

Since SKF alone did not alter infarct size, the

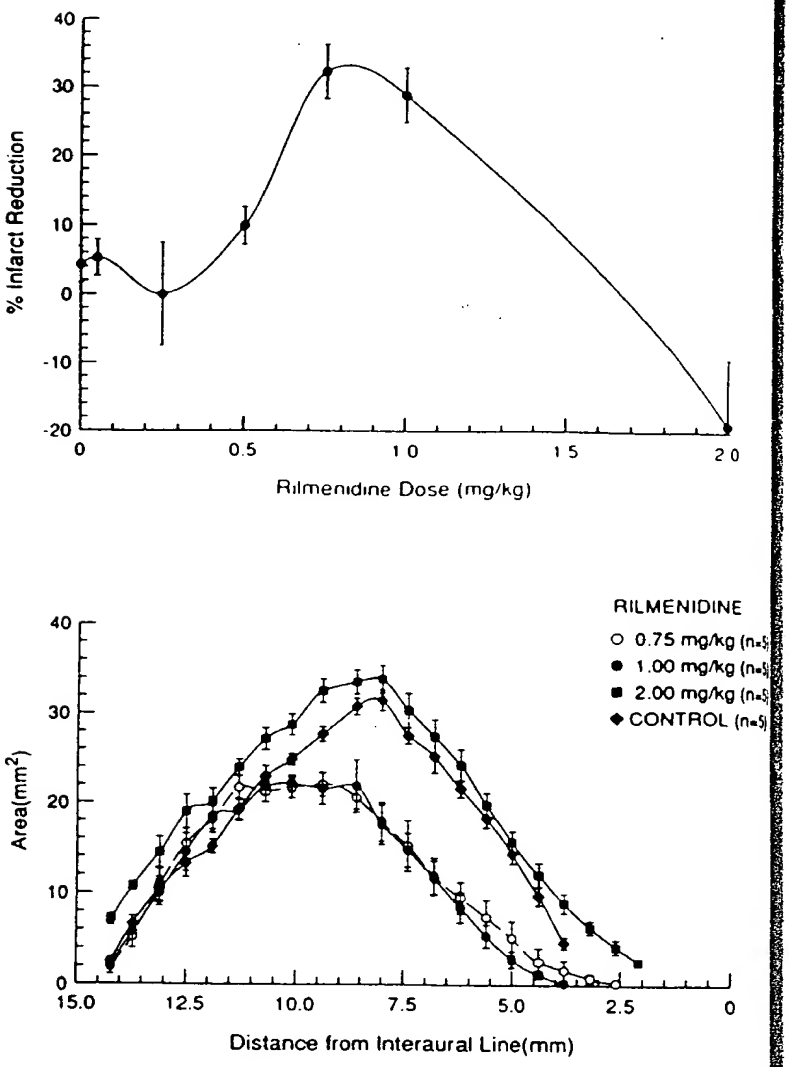

FIG. 3A. Effect of rilmenidine (RIL) dose on percentage infarct reduction. Each dose represents a group of five animals. Maximum protection occurs at 0.75 mg/kg. Infarct volume is increased by 19% with RIL 2.0 mg/kg. **3B.** Effect of RIL dose on distribution of ischemic infarct 24 h after occlusion of middle cerebral artery. Cross-sectional area of infarct (mm^2) was measured at progressive distances (mm) from the interaural line in control animals (n = 5) and animals treated with RIL (0.75 mg/kg, n = 5); 1.0 mg/kg, n = 5; 2.0 mg/kg, n = 5). Infarct area following administration of RIL 0.75 and 1.0 mg/kg was reduced in the caudal portion of the lesion. In contrast, administration of RIL 2.0 mg/kg increased infarct area in caudal as well as rostral portions of the lesion.

ability of IDA to reduce focal ischemia does not appear to be secondary to α_2-receptor antagonism. In an attempt to further define the mechanism of IDA's ability to salvage ischemic tissue, IDA (3 mg/kg) and SKF (15 mg/kg) were administered concurrently via intravenous bolus under the present experimental protocol. Although phenylephrine (4.5–11.5 μg/min) was able to counteract the hypotension from these agents during the 1-h protocol, animals did not survive the ensuing 24 h secondary to persistent hypotension (MABP 40 mm Hg).

Effects of elevating CBF

To assess whether the protective actions of RIL or IDA might relate to increasing CBF in the territory of tissue salvage, a region in which others have shown LCBF is substantially reduced (Dirnagl and Pulsinelli, 1990), LCBF was increased for the 1 h following occlusion of the MCA. LCBF was elevated in groups of five rats by either hypercapnia or hypertension. Changes in LCBF were monitored by LDF through a probe placed over the parietal cortex contralateral to MCA occlusion. Following MCA occlusion, animals in the hypercapnia group were ventilated with 5% CO_2 in 100% O_2, which elevated the Pco_2 to 66 mm Hg (Fig. 4, left; Table 2). LCBF rose immediately and was sustained for the hour at 197 ± 4% of control. The hypertension group received phenylephrine (0.5–2.0 μg/min) for 1 h following MCA occlusion, which elevated LCBF to 197 ± 4% of control and MABP to ~190 mm Hg, well above the upper limit of autoregulation in the SHR (Barry et al., 1982) (Fig. 4, left; Table 2).

LCBF elevated by hypercapnia did not alter infarct distribution or size. The MCA lesion of the hypercapnic group paralleled that of the control

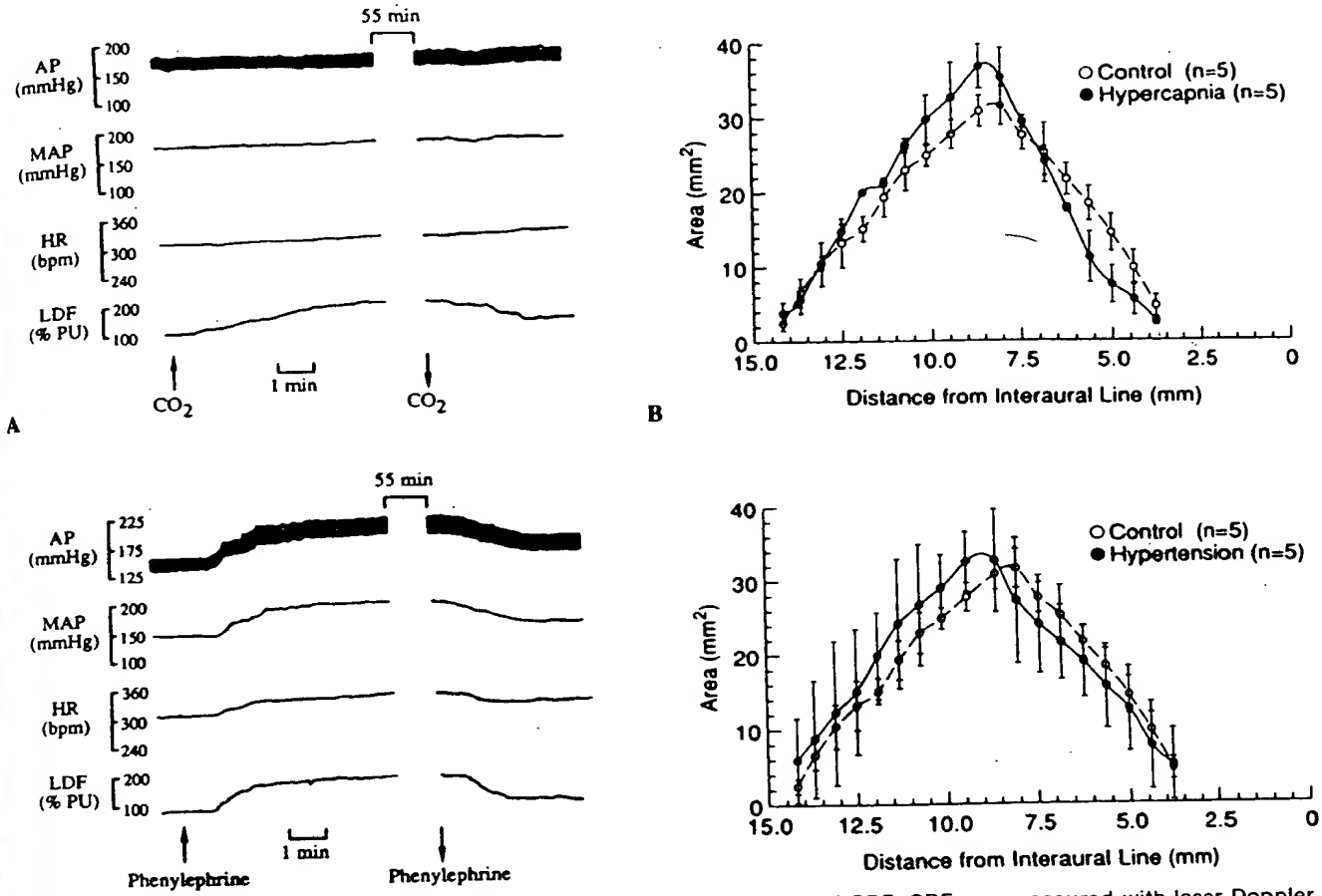

FIG. 4A. Effect of hypercapnia and hypertension on MABP, heart rate (HR), and CBF. CBF was measured with laser-Doppler flowmetry (LDF) over the parietal cortex contralateral from middle cerebral artery (MCA) infarction and expressed as percentage change of perfusion units (%ΔPU). Elevations in CBF of 200% of control were maintained for 1 h by hypercapnia (Pco_2 = 66.2 ± 8.2 mm Hg) or by hypertension with phenylephrine to MABP of 189 ± 9 mm Hg. **4B:** Effect of hypercapnia and hypertension on distribution of infarct 24 h following MCA occlusion. Cross-sectional area of infarct (mm^2) was measured along the rostro-caudal axis from the interaural line in control animals (n = 5) and animals with CBF elevated to 200% of control by either hypercapnia (n = 5) or hypertension (n = 5). Note that both methods of increased CBF failed to alter size or distribution of lesion.

group, extending 10.5 mm from the frontal pole to the temporal cortex. The maximum cross-sectional area of infarction was 36.98 ± 6.72 mm^2 and occurred ~9.0 mm anterior to the interaural line as in the control group (Fig. 4, right). The average infarct volume of the hypercapnic group was 201.34 ± 45.54 mm^3 and did not differ significantly from the average infarct volume of the control group (196.31 ± 14.86 mm^3) (Table 1).

Elevation on LCBF by hypertension also did not affect infarct distribution or size. Similar to the control group, the MCA lesion of the hypertensive group extended 10.5 mm from the frontal pole to the temporal cortex. The maximum cross-sectional area of infarction was 32.71 ± 6.95 mm^2 and occurred 9.0 mm anterior to the interaural line (Fig. 4, right). The average infarct volume of 203.96 ± 35.10 mm^3 in the hypertensive group was not significantly different from the infarct volume of 196.31 ± 14.86 mm^3 in the control group (Table 1).

DISCUSSION

Our results demonstrate that RIL and IDA can partially reduce the size of focal cerebral ischemic infarctions elicited by occlusion of the MCA in rat. RIL elicited a maximal reduction of ~33%, which was dose dependent. IDA also was protective but less effective, providing only a 22% reduction in lesion size. However, this finding demonstrates that IDA is effective as a neuroprotective agent not only in global (Gustafson et al., 1989) but also in focal cerebral ischemia.

The neuroprotective effects of IDA and RIL do not appear to be secondary to variations in blood

gases, hematocrit, or MABP or to the use of isoflurane as an anesthetic. The control and drug groups did not experience differences in ventilatory or hemodynamic function. Partial pressures for O_2 and CO_2 as well as values for pH and hematocrit did not differ significantly among the groups except when P_{CO_2} or MABP was deliberately elevated. In addition, hematocrit was maintained above the level at which hemodilution has been reported to reduce ischemic damage (Tu et al., 1988). Although IDA, RIL, and SKF all reduced MABP upon administration, MABP was maintained within the control range for the 1 h following occlusion of the MCA and then discontinued before cessation of the anesthesia. Following discontinuation of phenylephrine, however, the MABP for all groups, with one exception, returned to the control range. Thus, MABP cannot be considered a relevant variable. The exception comprised animals treated with the highest dose of RIL, 2 mg/kg. These animals were hypotensive at the termination of the 1 h following drug administration, which, as others have demonstrated (Cole et al., 1990), probably contributed to the enlargement of the lesion relative to that of the control group. Isoflurane administration is known to reduce infarct size during periods of focal cerebral ischemia (Baughman et al., 1990). Although we did not study the isolated role of isoflurane on the volume of cerebral infarction, control as well as drug treatment groups received identical doses of the anesthetic. Therefore, reductions in infarct volume following drug administration can be attributed to the neuroprotective effects of IDA and RIL.

The area of salvage provided by RIL and IDA included lesions of dorsal and ventral parietal cortex, temporal cortex, perirhinal cortex, pyriform cortex, lateral caudate-putamen, and dorsal frontal cortex. Preservation was more prominent in the caudal region of the hemisphere. In general, the area of salvage was comparable with that obtained by others in this focal ischemic model in rat using other cerebral protective agents such as calcium channel or *N*-methyl-D-aspartate antagonists (Roman et al., 1989; Jacewicz et al., 1990). This area most likely corresponds to the penumbral zone (Astrup et al., 1981) characterized by partial reduction in LCBF and patchy elevations in regional cerebral glucose utilization (Nedergaard et al., 1986) and may represent those regions of brain accessible to collateral flow.

The pharmacological basis of the neuroprotection afforded by RIL and IDA is not obvious. In recent series of studies, Gustafson et al. (1989, 1990) have suggested that the mechanism of IDA in protecting against neuronal death initiated by global ischemia relates to the action of the drug as an α_2-adrenergic antagonist. They propose that IDA, by presynaptically facilitating the release of noradrenaline from nerve endings in brain (Dennis et al., 1987) and/or, as others have demonstrated, by increasing the activity of noradrenergic neurons of the locus ceruleus (Simson and Weiss, 1987), enhances the accumulation of noradrenaline, which functions to preserve ischemic tissue. In addition, they note that reduction of the release of noradrenaline in the brain, either pharmacologically or by lesions of the locus ceruleus, enhances the neuronal damage produced by seizures or global ischemia (Blomqvist et al., 1985; Nevander et al., 1986), a position not entirely supported by other studies, which suggest the opposite (Welch et al., 1975; Fridovich, 1978).

However, it is difficult to reconcile their interpretation with our findings that RIL offers even greater protection than IDA. Unlike IDA, RIL is in part an α_2-adrenergic agonist. It reduces the release of noradrenaline prejunctionally in the periphery (Verbeuren et al., 1986; Li and Rand, 1988) and decreases the firing of locus ceruleus neurons (Dresse et al., 1988). Thus, with respect to the α_2-adrenergic receptor, the actions of RIL are opposite to those of IDA. Moreover, the fact that the selective α_2-adrenergic antagonist SKF (Hieble et al., 1986) had no effect upon the size of the infarction also argues against the theory that IDA reduces the size of ischemic infarctions by an action as an α_2-adrenergic antagonist. An alternative interpretation is that both RIL and IDA afford protection against ischemic infarctions by an interaction with a novel class of receptors that recognize a number of agents with an IM structure, the IM receptors.

The concept of the IM receptor arose from the recognition that the central hypotensive actions of clonidine and other centrally acting hypotensive agents appeared, on the basis of structure-function analysis, to relate more to their structure as substituted IMs than to their purported actions as α_2-adrenergic agonists (Bousquet et al., 1984; Ernsberger et al., 1987). This fact has been proved by ligand binding studies, which have demonstrated that clonidine and congeners bind not only to α_2-adrenergic binding sites but to a distinct membrane site recognizing IMs including IDA, some oxazoles such as RIL, some alkaloids such as yohimbine and some guanidinium compounds. Binding at these sites is saturable with high affinity, stereospecific and not displaced by substituted phenylethylamines such as the catecholamines (Meeley et al., 198(Ernsberger et al., 1987; Michel et al., 1989; Parini et al., 1989). Nor is the IM receptor a histamine receptor (Ernsberger et al., 1987), even though hist

mine is the principal bioactive endogenously synthesized IM. That the IM α_2-adrenergic receptors are in fact distinct entities has been demonstrated by the findings that they have a different topographic distribution in brain and kidney (Ernsberger et al., 1987; Bricca et al., 1989*a;* Ernsberger et al., 1990), that they may be expressed in different cell lines (Feinland et al., 1988; Ernsberger et al., 1989), and that their signal transduction mechanisms differ (Ernsberger et al., 1988*a;* Regunathan et al., 1990).

Within this context is the fact that IDA and RIL bind to IM as well as to α_2-adrenergic receptors (Bricca et al., 1989*a;* Gomez et al., 1991) but that their actions at the latter differ. Whether the agents act as complete or partial agonists or antagonists at these sites is not known. However, the demonstration here that IDA and RIL, with opposite actions on α_2-adrenergic receptors, share common neuroprotective actions suggests that the commonality of pharmacological mechanisms is an interaction with IM rather than with adrenergic receptors.

The mechanism by which IDA and RIL may enhance protection from focal ischemia is not known. Two broadly defined mechanisms should be considered. The first, a direct action, would involve agents acting within the target to provide protection. The second, an indirect action, would invoke IM receptor binding at a site remote from the target, which may influence the size of the infarction by a transneuronal pathway.

The direct actions of RIL and IDA in the target area could be via an enhancement of LCBF. Yet, it does not seem probable that the neuroprotective effects of IDA and RIL can be attributed solely to elevations in LCBF, conceivably related to actions of the agents upon extracerebral or pial vessels (Bevan et al., 1987). Doubling of LCBF in the normal hemisphere by hypercapnia or hypertension for 1 h following MCA occlusion did not alter the distribution or size of the infarction produced by MCA occlusion. While changes within the penumbra were not measured, it is probable that the flow changes would be enhanced, since autoregulation is partially impaired in that zone (Dirnagl and Pulsinelli, 1990). The failure of elevations of LCBF to modify the magnitude of the infarction is in accord with the conclusions of some (Fenske et al., 1975; Bleyarert et al., 1980) but not all (Hayashi et al., 1984; Drummond et al., 1988) that elevating LCBF does not afford protection from focal ischemia.

Another possibility is that the agents might act directly upon neurons in the salvageable zone within the penumbra to protect them from cytotoxic ionic and/or metabolic events as is believed to be the mechanism of *N*-methyl-D-aspartate receptor antagonists. However, it should be noted that binding studies have demonstrated that IM receptors are sparse in the bovine, rat, and human cerebral cortex (Ernsberger et al., 1987; Bricca et al., 1988, 1989*b*) in contrast to the brainstem where, at least in bovine brain, 30% of binding sites for clonidine are of the IM receptor subclass (Ernsberger et al., 1988*b*). Thus, the paucity of IM receptors within the cortex makes a direct cytoprotective action of the agents an unlikely mechanism, recognizing the absence of in vitro studies with respect to neuroprotection by RIL or IDA.

Conceivably the action of RIL and/or IDA could be indirect, in which the agents would act on neurons elsewhere in the brain to exert a neuroprotective effect on ischemic neurons over intrinsic pathways. In this scenario, activation (or inhibition) of the pathway would result in release of other transmitters in the target, which would be the neuroprotective moieties. The feasibility of this mechanism derives from recent studies demonstrating that electrical stimulation of the cerebellar fastigial nucleus can reduce infarct volume by 40% following MCA occlusion (Berger et al., 1990; Reis et al., 1991). Since the cerebrovascular effects of fastigial nucleus stimulation can be inhibited by bilateral lesions of the rostral ventrolateral medulla (Chida et al., 1990), a site rich in IM receptors (Bricca et al., 1988, 1989*b;* Ernsberger et al., 1988; Gomez et al., 1991), this area might be one site of action of the drug.

In conclusion, we have demonstrated that IDA and RIL significantly reduce the size of focal ischemic infarction elicited by MCA occlusion in rat. The effect cannot be attributed to α_2-antagonism by IDA, nor is it likely to be a consequence of the α_2-adrenergic agonism of RIL. Since IDA and RIL both bind to IM receptors, it is conceivable that occupation of these sites may be responsible for the neuroprotection of both agents. It is unknown whether the effects are due to direct actions of these agents on ischemic neurons in the target or indirect activation of neural networks that transneuronally modify cerebral ischemic injury.

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